Fig 1. Examples of two test sets of peptides and results with PKC-theta

A. P+1 test set

Position P+1 Symbolic Representation: ddddRdd-S-?dd								
	Peptide Sequence			CPM		Ratio to Mean	Log Score	
1	ddddRdd–S–	D	dd	67	± 3	0.4	-1.26	± 0.03
2	ddddRdd–S–	N	dd	88	± 3	0.5	-0.87	± 0.04
3	ddddRdd–S–	Q	dd	158	± 4	1.0	-0.03	± 0.04
4	ddddRdd–S–	R	dd	[illegible]	± 9	[illegible]	[illegible]	± 0.04
5	ddddRdd–S–	K	dd	[illegible]	± 18	[illegible]	[illegible]	± 0.08
6	ddddRdd–S–	H	dd	163	± 10	1.0	0.01	± 0.09
7	ddddRdd–S–	S	dd	136	± 3	0.8	-0.24	± 0.03
8	ddddRdd–S–		dd	90	± 3	0.6	-0.85	± 0.07
9	ddddRdd–S–	L	dd	[illegible]	± 18	[illegible]	[illegible]	± 0.11
10	ddddRdd–S–	F	dd	[illegible]	± 8	[illegible]	[illegible]	± 0.03
11	ddddRdd–S–	W	dd	135	± 6	0.8	-0.25	± 0.07
12	ddddRdd–S–	G	dd	93	± 2	0.6	-0.79	± 0.03
13	ddddRdd–S–	A	dd	97	± 5	0.6	-0.73	± 0.10

B. P+2 test set

Position P+2 Symbolic Representation: ddddRdd-S-F?d								
	Peptide Sequence			CPM		Ratio to Mean	Log Score	
1	ddddRdd–S–F	D	d	186	± 11	0.5	-1.12	± 0.08
2	ddddRdd–S–F	N	d	[illegible]	± 22	[illegible]	[illegible]	± 0.04
3	ddddRdd–S–F	Q	d	229	± 7	0.6	-0.83	± 0.05
4	ddddRdd–S–F	R	d	[illegible]	± 38	[illegible]	[illegible]	± 0.05
5	ddddRdd–S–F	K	d	[illegible]	± 25	[illegible]	[illegible]	± 0.05
6	ddddRdd–S–F	H	d	[illegible]	± 35	[illegible]	[illegible]	± 0.06
7	ddddRdd–S–F	S	d	431	± 10	1.1	0.09	± 0.05
8	ddddRdd–S–F		d	148	± 3	0.4	-1.46	± 0.03
9	ddddRdd–S–F	L	d	489	± 5	1.2	0.27	± 0.01
10	ddddRdd–S–F	F	d	543	± 19	1.3	0.42	± 0.07
11	ddddRdd–S–F	W	d	459	± 3	1.1	0.18	± 0.01
12	ddddRdd–S–F	G	d	195	± 2	0.5	-1.05	± 0.01
13	ddddRdd–S–F	A	d	201	± 7	0.5	-1.01	± 0.07

Fig 2: A superset of test sets designed for analysis of PKC specificity from P-4 to P+3

Symbolic Representation		ddd?R??–S–F??							
Anch r and ph sph rylatable Residues		Rxx–S–F					–S–F	Rxx–x–F	Rxx–S–
Set		Set P-4	Set P-2	Set P-1	Set P+2	Set P-3	Set P-3	Set P-2	Set P+1
P siti n		P–4	P–2	P–1	P+2	P+3	P–3	P0	P+1
Symbolic Representati n f Set		ddd?Rdd-S-Fdd	ddddR?d-S-Fdd	ddddRd?-S-Fdd	ddddRdd-S-F?d	ddddRdd-S-Fd?	dddd?dd-S-Fdd	ddddRdd-?-Fdd	ddddRdd-S-?dd
Res	ther residues in class								
D	E	dddDRdd-S-Fdd	ddddRDd-S-Fdd	ddddRdD-S-Fdd	ddddRdd-S-FDd	ddddRdd-S-FdD	ddddDdd-S-Fdd		ddddRdd-S-Ddd
		ddd Rdd-S-Fdd	ddddR d-S-Fdd	ddddRd -S-Fdd	ddddRdd-S-F d	ddddRdd-S-Fd	dddd dd-S-Fdd		ddddRdd-S- dd
		ddd Rdd-S-Fdd	ddddR d-S-Fdd	ddddRd -S-Fdd	ddddRdd-S-F d	ddddRdd-S-Fd	dddd dd-S-Fdd		ddddRdd-S- dd
R		dddRRdd-S-Fdd	ddddRRd-S-Fdd	ddddRdR-S-Fdd	ddddRdd-S-FRd	ddddRdd-S-FdR	ddddRdd-S-Fdd		ddddRdd-S-Rdd
K		dddKRdd-S-Fdd	ddddRKd-S-Fdd	ddddRdK-S-Fdd	ddddRdd-S-FKd	ddddRdd-S-FdK	ddddKdd-S-Fdd		ddddRdd-S-Kdd
H		dddHRdd-S-Fdd	ddddRHd-S-Fdd	ddddRdH-S-Fdd	ddddRdd-S-FHd	ddddRdd-S-FdH	ddddHdd-S-Fdd		ddddRdd-S-Hdd
S	T	dddSRdd-S-Fdd	ddddRSd-S-Fdd	ddddRdS-S-Fdd	ddddRdd-S-FSd	ddddRdd-S-FdS	ddddSdd-S-Fdd		ddddRdd-S-Sdd
P		dddPRdd-S-Fdd	ddddRPd-S-Fdd	ddddRdP-S-Fdd	ddddRdd-S-FPd	ddddRdd-S-FdP	ddddPdd-S-Fdd		ddddRdd-S-Pdd
L	IMV	dddLRdd-S-Fdd	ddddRLd-S-Fdd	ddddRdL-S-Fdd	ddddRdd-S-FLd	ddddRdd-S-FdL	ddddLdd-S-Fdd		ddddRdd-S-Ldd
F	Y	dddFRdd-S-Fdd	ddddRFd-S-Fdd	ddddRdF-S-Fdd	ddddRdd-S-FFd	ddddRdd-S-FdF	ddddFdd-S-Fdd		ddddRdd-S-Fdd
V		dddVRdd-S-Fdd	ddddRVd-S-Fdd	ddddRdV-S-Fdd	ddddRdd-S-FVd	ddddRdd-S-FdV	ddddVdd-S-Fdd		ddddRdd-S-Vdd
G		dddGRdd-S-Fdd	ddddRGd-S-Fdd	ddddRdG-S-Fdd	ddddRdd-S-FGd	ddddRdd-S-FdG	ddddGdd-S-Fdd		ddddRdd-S-Gdd
A		dddARdd-S-Fdd	ddddRAd-S-Fdd	ddddRdA-S-Fdd	ddddRdd-S-FAd	ddddRdd-S-FdA	ddddAdd-S-Fdd		ddddRdd-S-Add
S								ddddRdd-S-Fdd	
T								ddddRdd-T-Fdd	

Residues that vary within a query position

Fig 3: Raw Counts For In Vitro phosphorylation by PKC-theta of a collection of peptides designed for analysis of PKC specific

Residue	-4	-3	-2	-1	P0	+1	+2	+3
D	[illegible]	78	419	294		67	186	263
N	422	153	477	651		88	[illegible]	610
Q	411	[illegible]	493	472		158	229	358
R	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
K	[illegible]	117	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
H	573	117	[illegible]	461		163	[illegible]	506
S	433	90	389	371	461	136	431	304
	439	93	458	376		90	148	226
L	640	85	347	413		[illegible]	489	715
F	741	[illegible]	243	348		[illegible]	543	647
W	721	151	301	291		135	459	470
G	517	127	449	672		93	195	409
A	380	83	362	678		97	201	711
T					402			
Geo Mean For Set	572	133	509	481	430	161	406	488

Fig 4: Ratio to Mean for PKC-theta

Residue	-4	-3	-2	-1	P0	+1	+2	+3
D	0.5	0.6	0.8	0.6		0.4	0.5	0.5
N	0.7	1.1	0.9	1.4		0.5		1.3
Q	0.7		1.0	1.0		1.0	0.6	0.7
R								
K		0.9						
H	1.0	0.9		1.0		1.0		1.0
S	0.8	0.7	0.8	0.8	1.1	0.8	1.1	0.6
	0.8	0.7	0.9	0.8		0.6	0.4	0.5
L	1.1	0.6	0.7	0.9			1.2	1.5
F	1.3		0.5	0.7			1.3	1.3
W	1.3	1.1	0.6	0.6		0.8	1.1	1.0
G	0.9	1.0	0.9	1.4		0.6	0.5	0.8
A	0.7	0.6	0.7	1.4		0.6	0.5	1.5
T					0.9			

Fig 5: Position-specific scoring matrix for PKC-theta

	Residue	-4	-3	-2	-1	P0	+1	+2	+3
Experimentally determined	D	-1.0	-0.8	-0.3	-0.7		-1.3	-1.1	-0.9
	N	-0.4	0.2	-0.1	0.4		-0.9		0.3
	Q	-0.5		0.0	0.0		0.0	-0.8	-0.4
	R								
	K		-0.2						
	H	0.0	-0.2		-0.1		0.0		0.1
	S	-0.4	-0.6	-0.4	-0.4	0.1	-0.2	0.1	-0.7
		-0.4	-0.5	-0.2	-0.4		-0.8	-1.5	-1.1
	L	0.2	-0.7	-0.6	-0.2			0.3	
	F	0.4		-1.1	-0.5			0.4	0.4
	W	0.3	0.2	-0.8	-0.7		-0.3	0.2	-0.1
	G	-0.1	-0.1	-0.2	0.5		-0.8	-1.1	-0.3
	A	-0.6	-0.7	-0.5	0.5		-0.7	-1.0	
	T					-0.1			
Extrapolated	T	-0.4	-0.6	-0.4	-0.4		-0.2	0.1	-0.7
	E	-1.0	-0.8	-0.3	-0.7		-1.3	-1.1	-0.9
	I	0.2	-0.7	-0.6	-0.2		0.6	0.3	0.6
	M	0.2	-0.7	-0.6	-0.2		0.6	0.3	0.6
	V	0.2	-0.7	-0.6	-0.2		0.6	0.3	0.6
	Y	0.4	0.9	-1.1	-0.5		1.5	0.4	0.4
	C	-0.6	-0.7	-0.5	0.5		-0.7	-1.0	0.6

Fig 6. A superset of degenerate peptides designed to extend analysis of PKC specificity

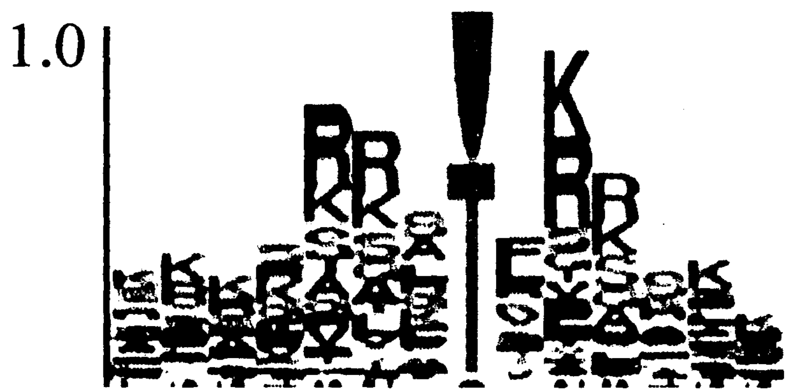
Sup rs t:			Superset A					
Anch r d R sidu s			Rxx–S–F					
S t			Set P-7	Set P-6	Set P-5	Set P+4	Set P+5	S t P+6
Position			P−7	P−6	P−5	P+4	P+5	P+6
Symbolic Repr s ntation of Set			dddd?Rdd-S-Fdd	ddddR?d-S-Fdd	dddddRd?-S-Fdd	ddRdd-S-F?dddd	ddRdd-S-F?dddd	dddRdd-S-Fd?dd
Residu s that vary within a variable position	R s	Other residues in class						
	D	E	9D999R99-S-F99	99D99R99-S-F99	999D9R99-S-F99	99R99-S-F99D99	99R99-S-F999D9	99R99-S-F9999D
	N		9N999R99-S-F99	99N99R99-S-F99	999N9R99-S-F99	99R99-S-F99N99	99R99-S-F999N9	99R99-S-F9999N
	Q		9Q999R99-S-F99	99Q99R99-S-F99	999Q9R99-S-F99	99R99-S-F99Q99	99R99-S-F999Q9	99R99-S-F9999Q
	R		9R999R99-S-F99	99R99R99-S-F99	999R9R99-S-F99	99R99-S-F99R99	99R99-S-F999R9	99R99-S-F9999R
	K		9K999R99-S-F99	99K99R99-S-F99	999K9R99-S-F99	99R99-S-F99K99	99R99-S-F999K9	99R99-S-F9999K
	H		9H999R99-S-F99	99H99R99-S-F99	999H9R99-S-F99	99R99-S-F99H99	99R99-S-F999H9	99R99-S-F9999H
	S	T	9S999R99-S-F99	99S99R99-S-F99	999S9R99-S-F99	99R99-S-F99S99	99R99-S-F999S9	99R99-S-F9999S
			9 999R99-S-F99	99 99R99-S-F99	999 9R99-S-F99	99R99-S-F99 99	99R99-S-F999 9	99R99-S-F9999
	L	IMV	9L999R99-S-F99	99L99R99-S-F99	999L9R99-S-F99	99R99-S-F99L99	99R99-S-F999L9	99R99-S-F9999L
	F	Y	9F999R99-S-F99	99F99R99-S-F99	999F9R99-S-F99	99R99-S-F99F99	99R99-S-F999F9	99R99-S-F9999F
	V		9V999R99-S-F99	99V99R99-S-F99	999V9R99-S-F99	99R99-S-F99V99	99R99-S-F999V9	99R99-S-F9999V
	G		9G999R99-S-F99	99G99R99-S-F99	999G9R99-S-F99	99R99-S-F99G99	99R99-S-F999G9	99R99-S-F9999G
	A		9A999R99-S-F99	99A99R99-S-F99	999A9R99-S-F99	99R99-S-F99A99	99R99-S-F999A9	99R99-S-F9999A

Fig 7. Extension of Position-specific scoring matrix for PKC-theta

	Residue	-7	-6	-5	+4	+5	+6
Experimentally determined	D	-0.9	-0.5	-0.9	-0.3	-0.4	-0.4
	E						
	N	0.1	-0.1	-0.2	-0.1	[illegible]	0.1
	Q	-0.2	0.0	-0.2	-0.2	0.0	-0.3
	R	[illegible]	[illegible]	[illegible]	[illegible]	0.0	[illegible]
	K	0.4	[illegible]	[illegible]	[illegible]	0.5	[illegible]
	H	-0.1	-0.1	0.1	[illegible]	0.2	0.0
	S	0.0	0.1	0.4	0.4	-0.2	0.1
	T						
		0.5	0.3	-0.2	0.0	0.0	0.1
	L	0.0	-0.5	0.5	-0.6	0.3	-0.3
	M						
	F	0.0	-0.3	0.1	-0.3	-0.1	0.1
	Y						
	W	-0.9	-0.4	-0.5	-0.8	-0.5	-1.1
	G	0.4	0.1	-0.2	0.0	0.0	0.1
	A	0.0	-0.2	-0.2	-0.3	-0.4	0.1

Fig 8. Sequence Logo vs PSSM Logo

Published Sequence Logo for PKC



PSSM Logo for PKC-theta from -7 to +6

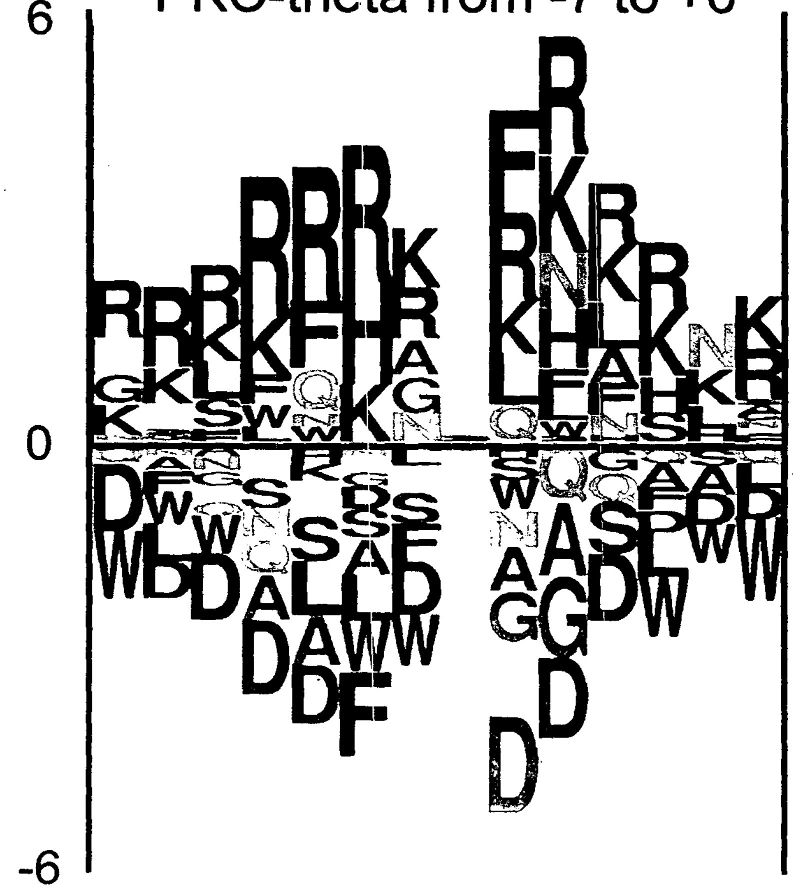


Fig 9. Testing our predictions for PKC-theta and Scansite prediction for PKC-delta against results for PKC-delta

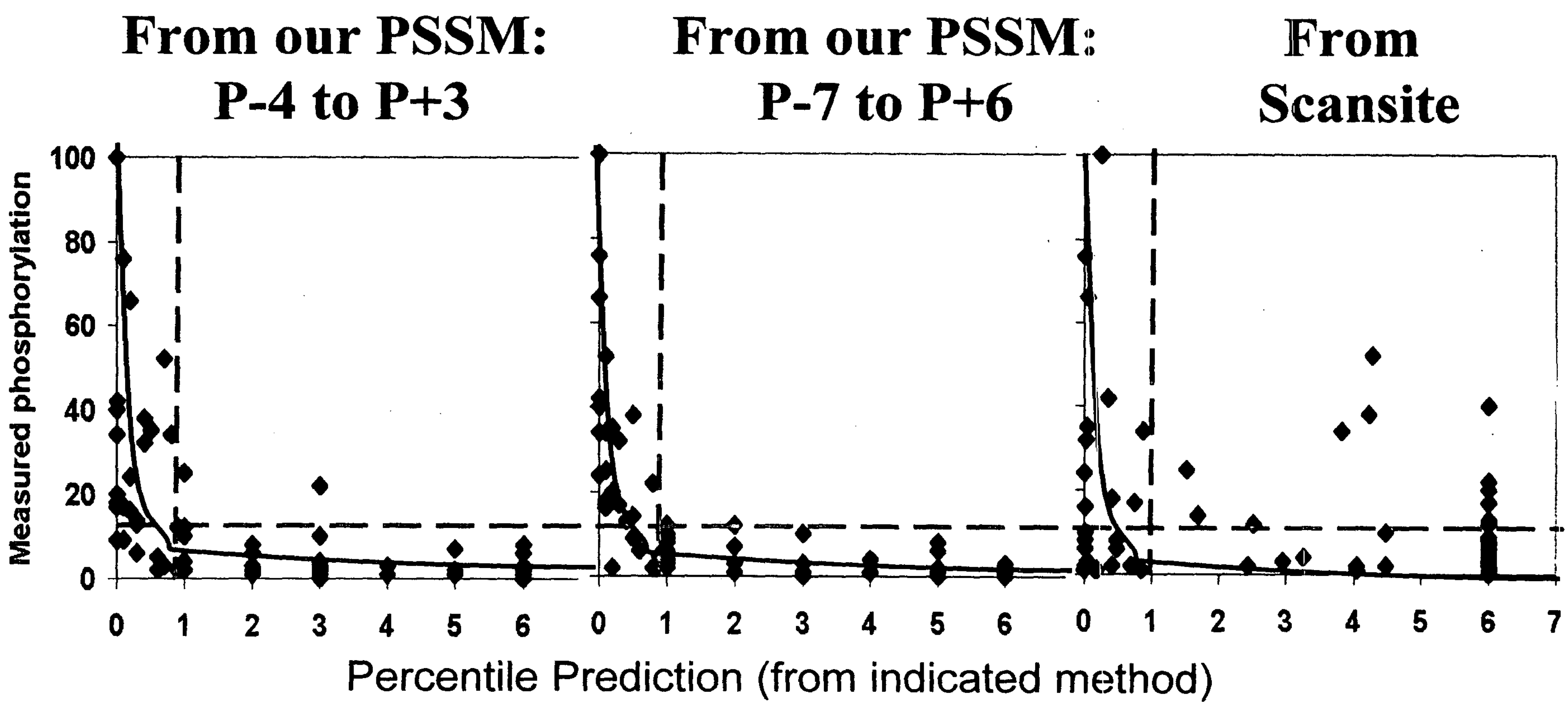


Fig 10: Comparison of our scoring with Scansite scoring for PKC-delta

Criteria:

Positive score: better than 1st percentile

Positive phosphorylation: better than 10% of max

Ours

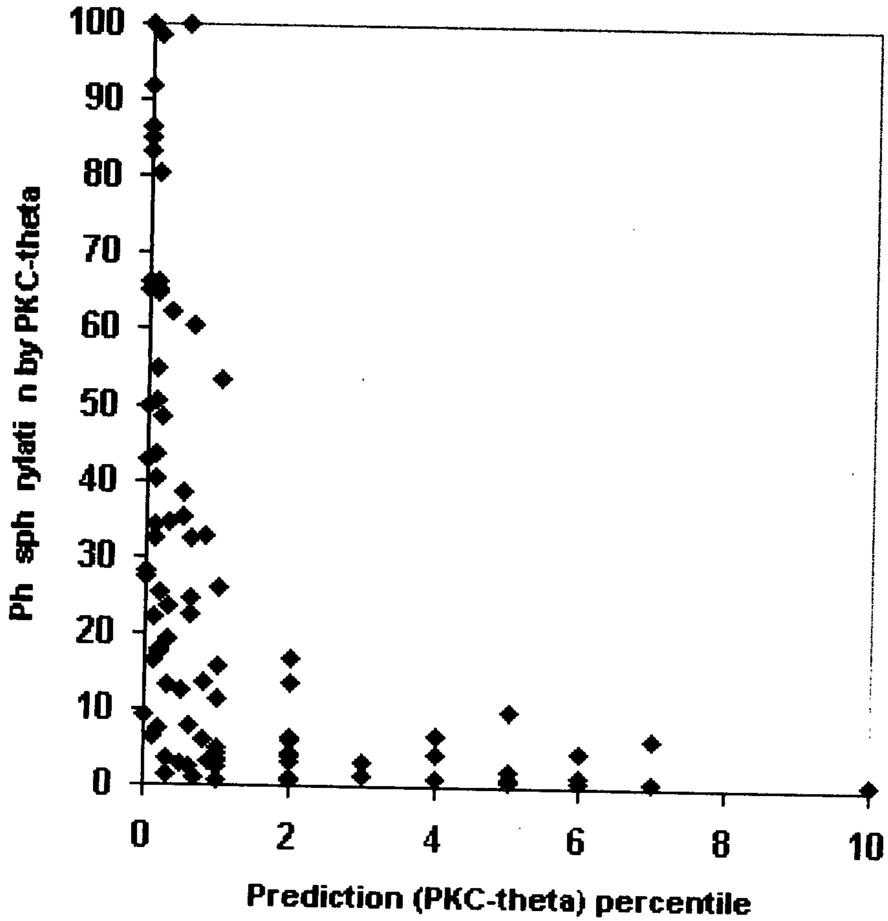
		Measured activity	
		Positive	Negative
Prediction score	Positive	21	4
	Negative	2	48
Total Scored		75	
Sensitivity		92	
Specificity		91	

Scansite

		Measured activity	
		Positive	Negative
Prediction score	Positive	10	16
	Negative	12	37
Total Scored		75	
Sensitivity		70	
Specificity		45	

Fig 11. Prospective validation of scoring for PKC-theta PSSM

A



B

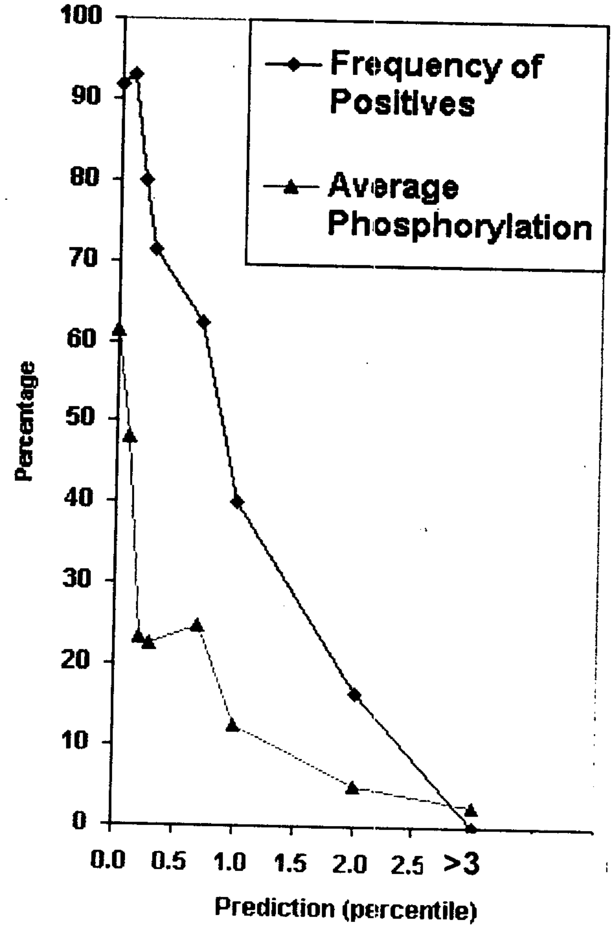


Fig 12. The d??R??S????d superset of test sets with 1 anchor position

Symbolic Representation		d??R??–S–????d							
Anchor and phosphorylatable Residues		Rxx–S							
Set		Set P-5	Set P-4	Set P-2	Set P-1	Set P+1	Set P-2	Set P+3	Set P+4
Position		P–5	P–4	P–2	P–1	P+1	P–2	P+3	P+4
Symbolic Representation of Set		d?dRdd-S-ddddd	dd?Rdd-S-ddddd	dddR?d-S-ddddd	dddRd?-S-ddddd	dddRdd-S-?dddd	dddRdd-S-d?ddd	dd?Rdd-S-dd?dd	ddd?Rdd-S-ddd?d
Residues that vary within a variable position Res	Other residues in class								
R		dRdRdd-S-ddddd	ddRRdd-S-ddddd	dddRRd-S-ddddd	dddRdR-S-ddddd	dddRdd-S-Rdddd	dddRdd-S-dRddd	dddRdd-S-ddRdd	dddRdd-S-dddRd
K		dKdRdd-S-ddddd	ddKRdd-S-ddddd	dddRKd-S-ddddd	dddRdK-S-ddddd	dddRdd-S-Kdddd	dddRdd-S-dKddd	dddRdd-S-ddKdd	dddRdd-S-dddKd
H		dHdRdd-S-ddddd	ddHRdd-S-ddddd	dddRHd-S-ddddd	dddRdH-S-ddddd	dddRdd-S-Hdddd	dddRdd-S-dHddd	dddRdd-S-ddHdd	dddRdd-S-dddHd
D		dDdRdd-S-ddddd	ddDRdd-S-ddddd	dddRDd-S-ddddd	dddRdD-S-ddddd	dddRdd-S-Ddddd	dddRdd-S-dDddd	dddRdd-S-ddDdd	dddRdd-S-dddDd
E		dEdRdd-S-ddddd	ddERdd-S-ddddd	dddREd-S-ddddd	dddRdE-S-ddddd	dddRdd-S-Edddd	dddRdd-S-dEddd	dddRdd-S-ddEdd	dddRdd-S-dddEd
		dNdRdd-S-ddddd	ddNRdd-S-ddddd	dddRNd-S-ddddd	dddRdN-S-ddddd	dddRdd-S-Ndddd	dddRdd-S-dNddd	dddRdd-S-ddNdd	dddRdd-S-dddNd
		dQdRdd-S-ddddd	dd Rdd-S-ddddd	dddR d-S-ddddd	dddRd -S-ddddd	dddRdd-S- dddd	dddRdd-S-dQddd	dddRdd-S-ddQdd	dddRdd-S-dddQd
F		dFdRdd-S-ddddd	ddFRdd-S-ddddd	dddRFd-S-ddddd	dddRdF-S-ddddd	dddRdd-S-Fdddd	dddRdd-S-dFddd	dddRdd-S-ddFdd	dddRdd-S-dddFd
Y		dYdRdd-S-ddddd	ddYRdd-S-ddddd	dddRYd-S-ddddd	dddRdY-S-ddddd	dddRdd-S-Ydddd	dddRdd-S-dYddd	dddRdd-S-ddYdd	dddRdd-S-dddYd
I	LV	dIdRdd-S-ddddd	ddIRdd-S-ddddd	dddRId-S-ddddd	dddRdI-S-ddddd	dddRdd-S-Idddd	dddRdd-S-dIddd	dddRdd-S-ddIdd	dddRdd-S-dddId
M		dMdRdd-S-ddddd	ddMRdd-S-ddddd	dddRMd-S-ddddd	dddRdM-S-ddddd	dddRdd-S-Mdddd	dddRdd-S-dMddd	dddRdd-S-ddMdd	dddRdd-S-dddMd
		d dRdd-S-ddddd	dd Rdd-S-ddddd	dddR d-S-ddddd	dddRd -S-ddddd	dddRdd-S- dddd	dddRdd-S-d ddd	dddRdd-S-dd dd	dddRdd-S-ddd d

Fig 13. PSSM Logo from analysis of d??R??S????d superset of peptides with AKT1

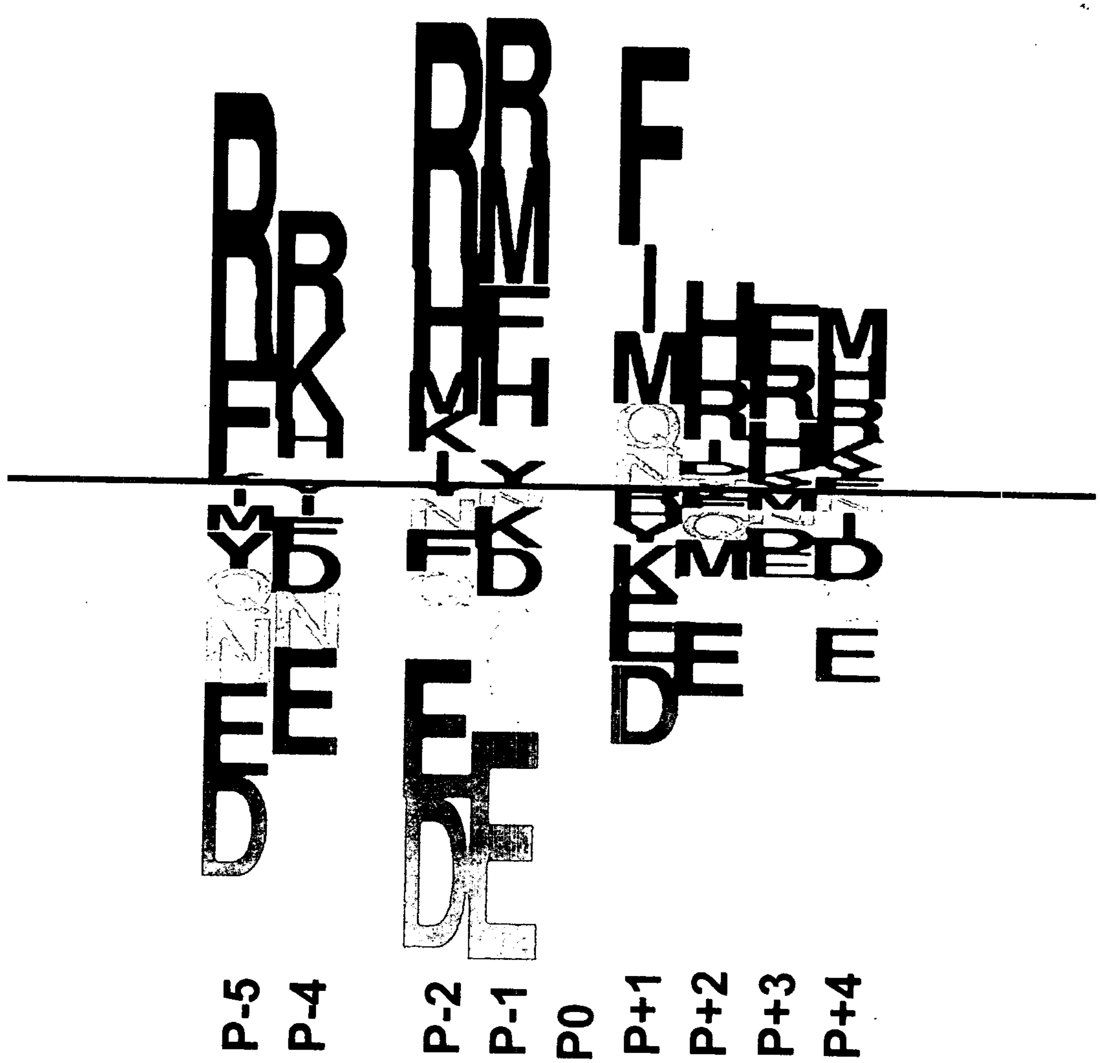


Fig 14: Issues related to abundance of residues in degenerate positions

Amino Acid		Target percentages					
		Plan 1	Plan 2	Plan 3			
Code	Name	Equal Abundance	Abundance In Human Proteome	Abundance in PKC substrates	Estimated incorporation efficiency	Reagent parts per hundred	Percent by sequencing
A	Alanine	5	7.1	6.6	1.1	8	15.8
C	Cysteine	5	2.4	0.8		0	
D	Aspartic	5	4.5	5.8	1.0	6	5.9
E	Glutamic	5	6.8	7.3	1.2	6	6.1
F	Phenylalanin	5	3.6	3.2	1.2	4	3.8
G	Glycine	5	6.9	7	1.0	8	9.2
H	Histidine	5	2.7	1.7	0.9	4	2.7
I	Isoleucine	5	4.2	3.3	0.7	4	1.6
K	Lysine	5	5.7	7.1	1.1	6	8.9
L	Leucine	5	9.8	6.8	0.9	6	9.1
M	Methionine	5	2.2	1.9	1.1	2	2.7
N	Asparagine	5	3.5	2.9	1.0	4	3.0
P	Proline	5	6.5	7.6	1.0	6	3.7
Q	Glutamine	5	4.8	2.8	1.2	6	3.9
R	Arginine	5	5.8	8.9	1.1	6	5.9
S	Serine	5	8.4	12.2	1.1	8	4.6
T	Threonine	5	5.4	5.9	0.8	6	3.1
V	Valine	5	5.9	4.3	0.8	4	3.5
W	Tryptophane	5	1.3	0.3		2	0.2
Y	Tyrosine	5	2.5	3.6	0.9	4	6.2

FIG. 15: Detection of specific phosphorylation of SHP-1 by Western blot with pPKC antibody which is augmented following stimulation by the T-cell receptor

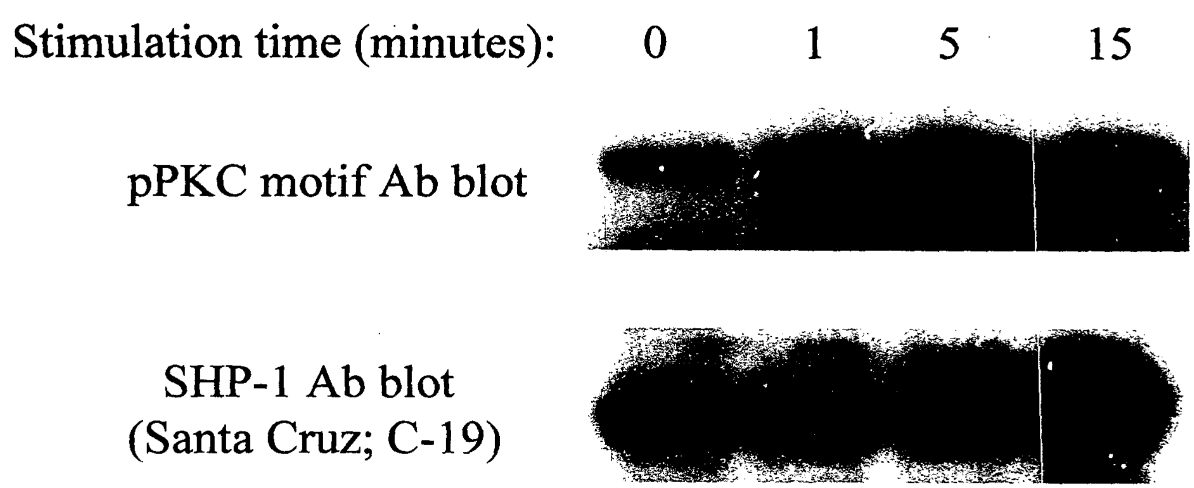


FIG. 16: Binding of pPKC antibody to phosphopeptides corresponding to those sites

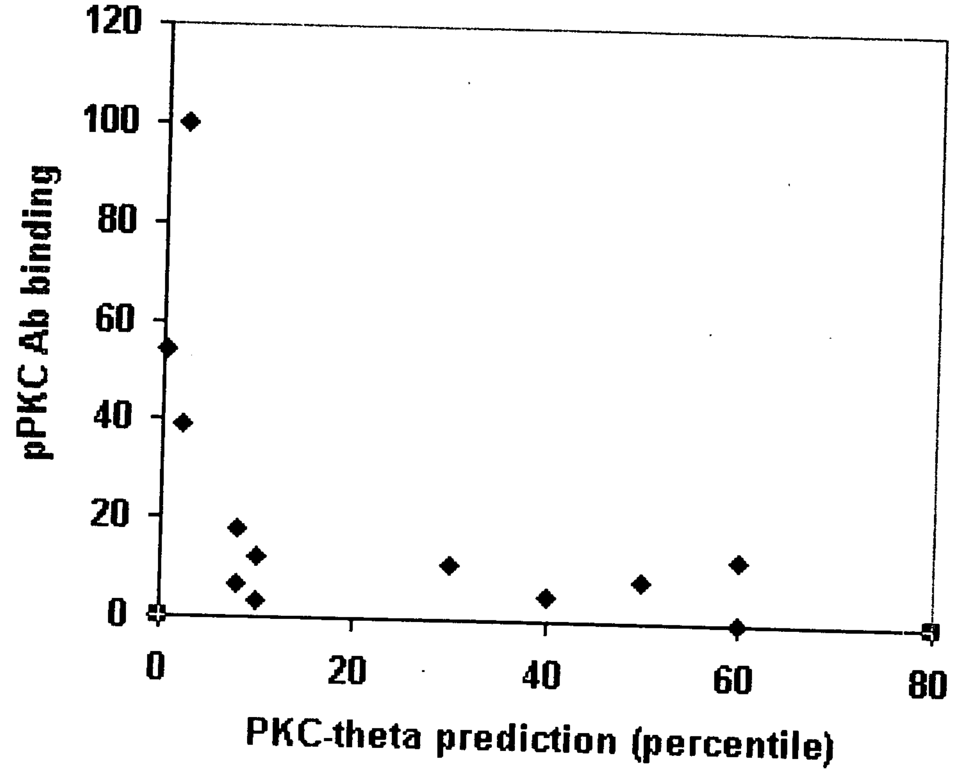


FIG. 17: Scores derived from different test sets tested at different times are reproducible and scores extrapolated for untested residues can be adequate

	Residue	Test Set #1	Test Set #2
Experimentally determined	D	-1.3	-1.3
	E		-0.9
	N	-0.9	-0.5
	C	0.0	0.5
	R	[illegible]	[illegible]
	K	[illegible]	[illegible]
	H	0.0	-0.2
	S	-0.2	-0.3
	T		0.2
		-0.8	-1.0
	L	[illegible]	0.7
	M		[illegible]
	F	[illegible]	1.4
	Y		[illegible]
	W	-0.3	-0.4
	G	-0.8	-0.7
	A	-0.7	-0.6

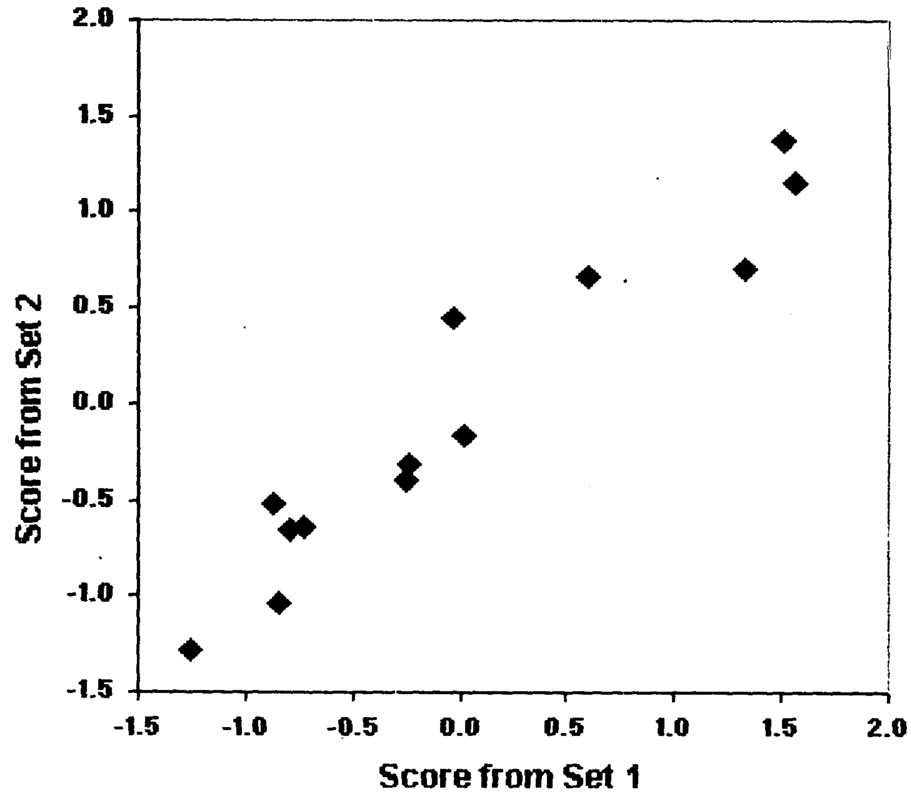


Fig 18: Scoring a peptide

Protein	Sequence	P0 Central	-7	-6	-5	-4	-3	-2	-1	P0	+1	+2	+3	+4	+5	+6	Score total	Percentile
MARCKS	KKKKKRF-S-FKKSFK	159	K [illegible]	K [illegible]	K [illegible]	K [illegible]	K -0.2	R [illegible]	F -0.5	S 0.1	F [illegible]	K [illegible]	K [illegible]	S [illegible]	F -0.1	K [illegible]	7.4	0.0

Fig 19. Distribution of PKC scores sites in proteins encoded by 15651 human genes

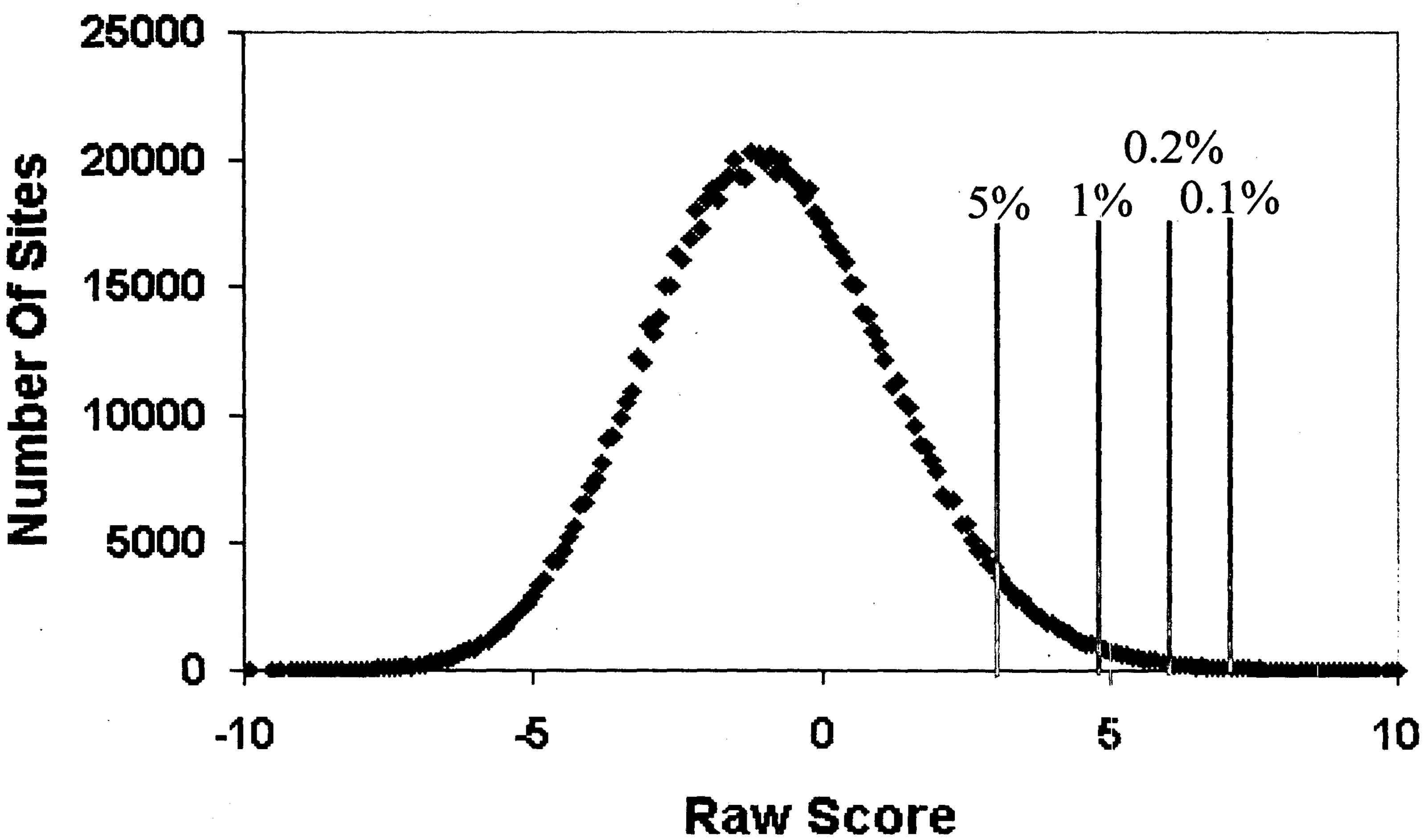
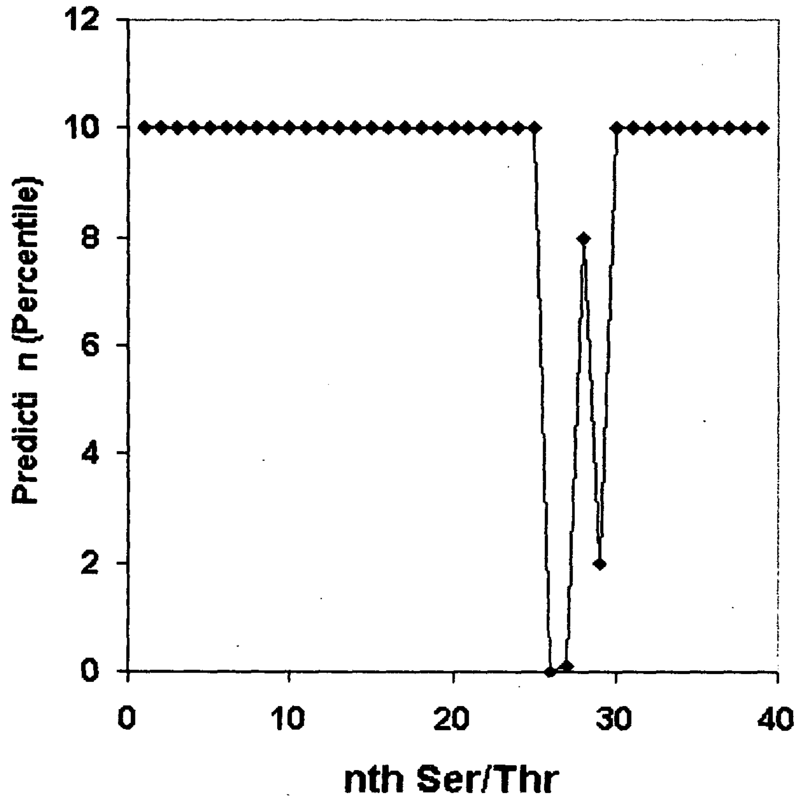


Fig 20: The PKC site prediction algorithm correctly predicts sites on MARCKS



Order	Sequence	P0 Residue Number	Percentile Predictions PKC Theta	AKT1	PKA
23	KAEDGATP-S-PSNETPKK	145	90	40	100
24	EDGATPSP-S-NETPKKKK	147	90	70	80
25	ATPSPSNE-T-PKKKKKRF	150	10	80	100
26	PKKKKKRF-S-FKKSFKLS	159	[redacted]	0.9	10
27	KKRFSFKK-S-FKLSGFSF	163	[redacted]	10	10
28	SFKKSFKL-S-GFSFKKNK	167	8	70	10
29	KSFKLSGF-S-FKKNKKEA	170	2	20	90
30	AAAEAGAA-S-GEQAAAPG	211	100	40	80
31	VAPEKPPA-S-DETKAAEE	252	70	40	80

FIG. 21. High similarity in specificity between novel and classical PKC isoforms, but atypical PKC differs more and great divergence seen with AKT1 and PKA

	Correlation (arithmetic)					
	alpha	delta	epsilon	zeta	AKT1	PKA
alpha	[illegible]	0.86	0.89	0.69	0.38	0.29
delta	0.86	[illegible]	[illegible]	0.73	0.63	0.35
epsilon	0.89	[illegible]	1.00	0.76	0.50	0.38
zeta	0.69	0.73	0.76	1.00	0.35	0.28
AKT1	0.38	0.63	0.50	0.35	1.00	0.51
PKA	0.29	0.35	0.38	0.28	0.51	1.00

Fig 22. Differences between PSSM Logos of different kinases analyzed with the same peptide collections

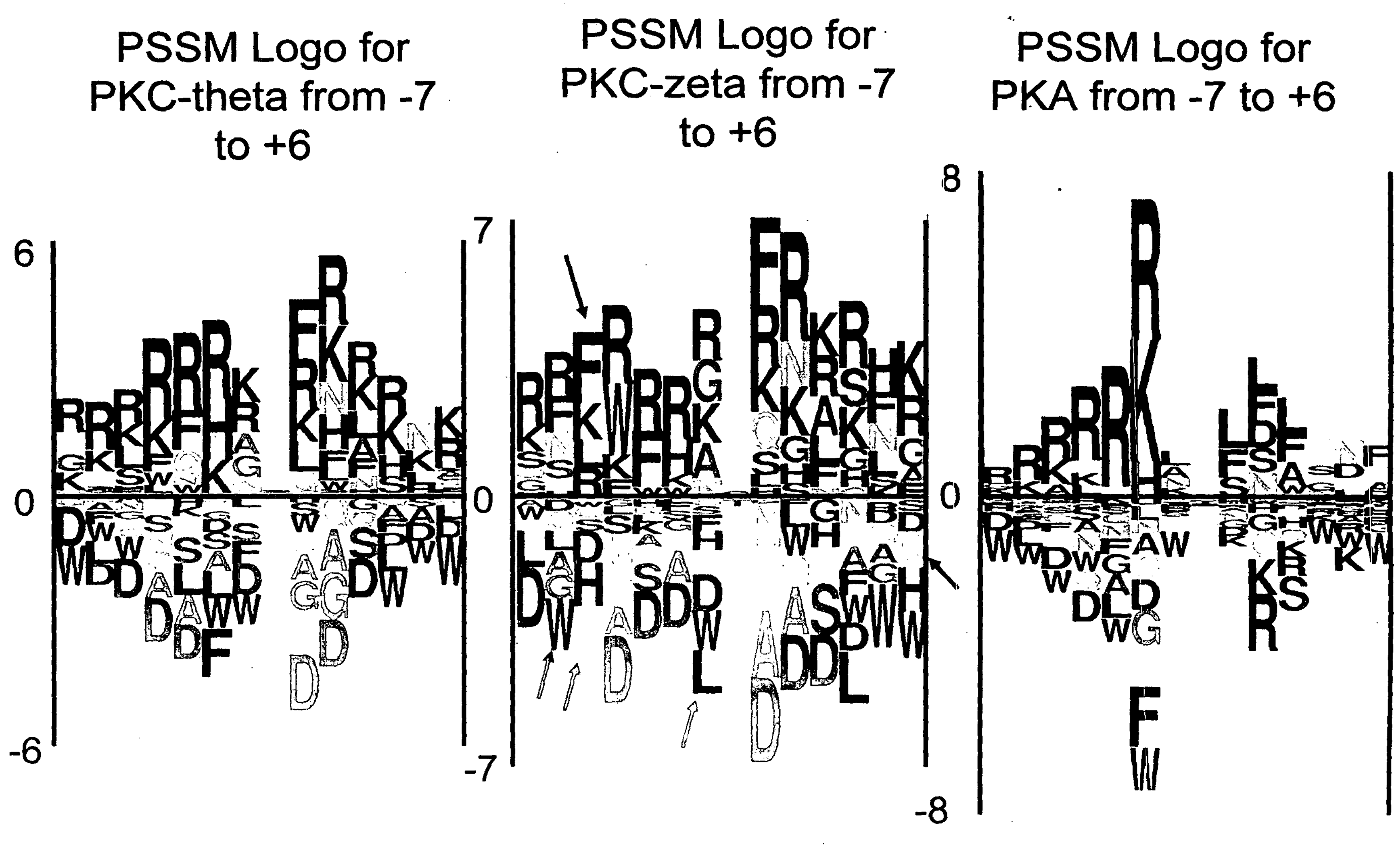


Fig 23. Prediction for PKC-zeta predicts PKC-zeta better than PKC-delta

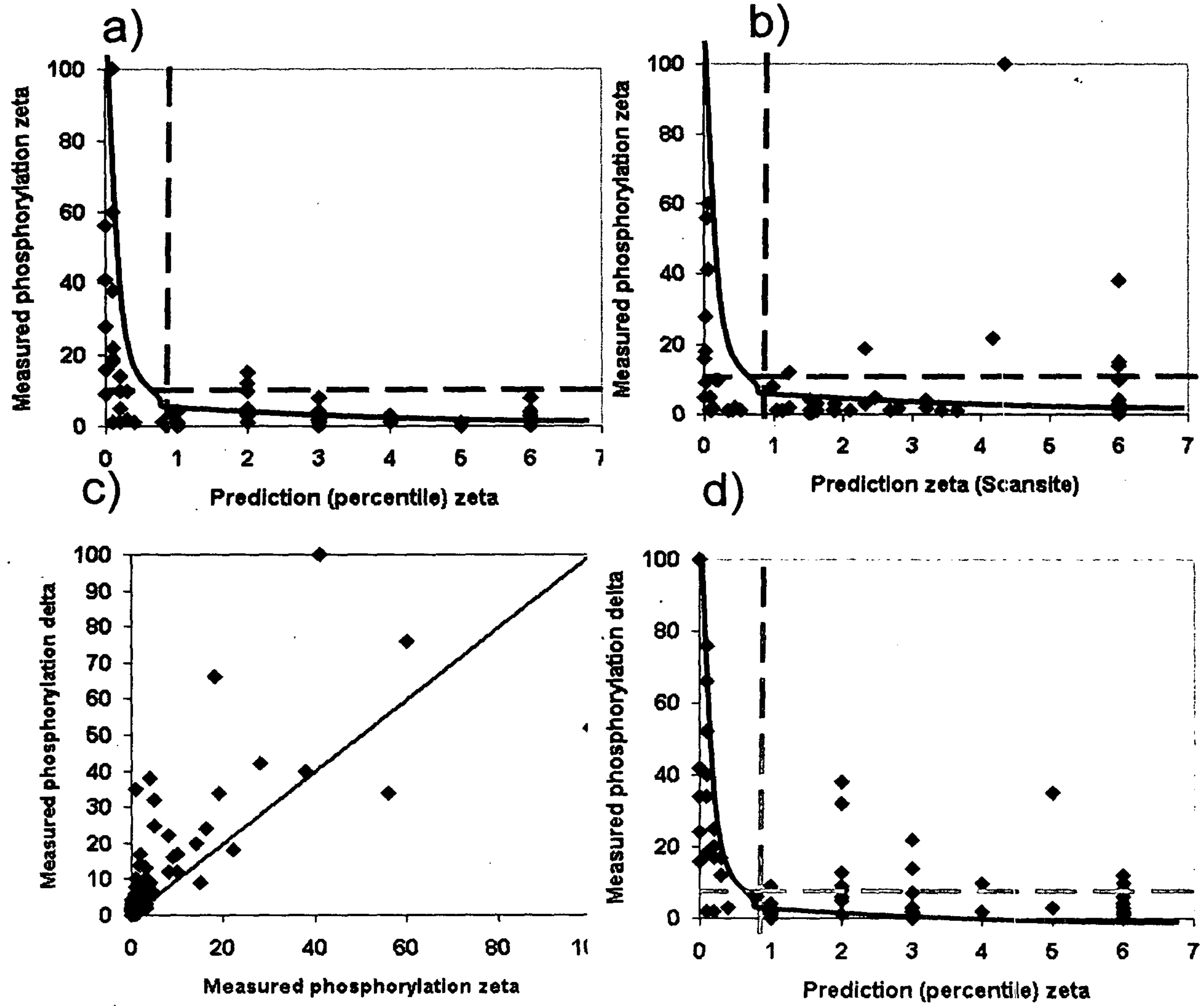


FIG. 24. Scoring changes in peptides that are less phosphorylated by PKC-zeta than PKC-delta

Peptide		Measured phosphorylation	Predicted percentile	-7	-6	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	+6
1				A	A	R	K	K	R	I	S	V	K	K	K	O	E
	difference	34	-4.8	0.1	0.4	0.2	0	0	-0.1	[illegible]	-0.1	0.3	-0.6	-0.7	-0.1	0.5	0.1
	zeta	1	5.0	-0.1	-0.6	0.7	0.8	-0.2	1.9	-1.6	0.2	0.4	1.9	1.5	1.0	-0.5	-0.5
	delta	35	[illegible]	0.0	-0.2	0.9	0.8	-0.2	1.8	-0.2	0.1	0.7	1.3	0.8	0.9	0.0	-0.4
2				K	I		K	R		G	S	V	H	R	T		S
	difference	34	-1.5	-0.2	0.1	[illegible]	0	-0.7	0.4	-1	-0.1	0.3	-0.3	-0.5	-0.6	0.2	[illegible]
	zeta	4	2.0	0.6	-0.6	-1.4	0.8	2.5	-0.6	1.5	0.2	0.4	1.1	1.3	1.0	-0.2	-0.4
	delta	38	0.5	0.4	-0.5	-0.2	0.8	1.8	-0.2	0.5	0.1	0.7	0.8	0.8	0.4	0.0	0.2
3				G	A			R	R	S	S	I	R	N	A	H	
	difference	10	-1.6	0.1	0.4	[illegible]	0.3	-0.7	-0.1	-0.3	-0.1	0.3	-2	0	0.2	-0.8	
	zeta	3	2.0	0.3	-0.6	-1.4	-0.7	2.5	1.9	-0.1	0.2	0.4	3.6	0.3	-0.5	1.0	
	delta	13	0.4	0.4	-0.2	-0.2	-0.4	1.8	1.8	-0.4	0.1	0.7	1.6	0.3	-0.3	0.2	
4				A	G	S	F	K	R	[illegible]	S	I	K	K	I	V	
	difference	12	-2.5	0.2	[illegible]	0.9	0.0	0.1	0.2	-0.1	-0.1	0.4	0.1	-0.3	[illegible]	-0.3	
	zeta	2	3.0	-0.2	-0.6	-0.5	0.4	-0.3	1.6	0.5	0.2	0.3	1.2	1.1	-1.3	0.6	
	delta	14	0.5	0.0	0.1	0.4	0.4	-0.2	1.8	0.4	0.1	0.7	1.3	0.8	-0.6	0.3	
5					W	K	G	K	R	R	S	K	A	R	K	K	R
	difference	14	-2.2		0.9	-0.4	-0.1	0	-0.1	-0.9	-0.1	-0.3	0.6	-0.5	-0.1	0.2	-0.3
	zeta	8	3.0		-1.3	0.9	-0.1	-0.2	1.9	1.6	0.2	1.0	-1.6	1.3	1.0	0.3	1.0
	delta	22	0.8		-0.4	0.5	-0.2	-0.2	1.8	0.7	0.1	0.7	-1.0	0.8	0.9	0.5	0.7
6				[illegible]		I	V	K	R	A	S	L	K	R	G	K	
	difference	27	-1.7	0.1	0.5	-0.3	0.3	0	-0.1	-0.7	-0.1	0.3	-0.6	-0.5	-0.6	0.2	0.6
	zeta	5	2.0	-0.1	-0.5	0.8	-0.1	-0.2	1.9	1.2	0.2	0.4	1.9	1.3	0.6	0.3	-0.9
	delta	32	0.3	0.0	0.0	0.5	0.2	-0.2	1.8	0.5	0.1	0.7	1.3	0.8	0.0	0.5	-0.3

FIG. 25 Position-specific residue preference for PKA and PKG determined using the PKC superset

PKA

	-4	-3	-2	-1	P0	+1	+2	+3
D	0.6	1.0	0.6	0.9		1.0	[illegible]	0.9
N	0.7	0.8	0.7	1.4		1.0	1.5	1.2
Q	0.7	0.8	0.6	1.0		0.9	0.6	0.8
R	[illegible]	[illegible]	[illegible]	1.1		1.2	0.5	0.7
K	1.3	1.1	[illegible]	1.1		1.0	0.5	0.8
H	1.1	0.9	[illegible]	0.9		1.1	0.8	0.8
S	0.8	1.1	0.9	0.9	0.9	1.0	[illegible]	0.6
P	1.3	1.2	0.5	0.8		0.3	1.1	0.9
L	1.2	0.6	0.7	1.3		[illegible]	[illegible]	[illegible]
F	1.1	0.7	0.3	1.0		[illegible]	[illegible]	[illegible]
W	0.7	0.6	0.3	0.6		1.2	1.0	1.2
G	0.9	0.7	0.9	1.0		0.9	0.7	1.0
A	0.8	0.7	0.6	1.2		0.9	1.0	[illegible]
T					1.1			

PKG

	-4	-3	-2	-1	P0	+1	+2	+3
D	0.4	1.1	0.6	0.4		0.7	[illegible]	0.9
N	0.4	1.3	0.5	[illegible]		0.8	1.4	1.2
Q	0.6	1.0	0.9	1.2		0.7	0.3	0.8
R	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	0.4	0.9
K	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	0.4	0.8
H	1.3	1.0	0.6	1.5		0.7	1.2	0.2
S	0.6	0.9	1.0	0.8	0.9	0.8	[illegible]	0.7
P	1.0	0.9	0.6	0.5		0.5	0.5	0.9
L	1.1	0.6	0.5	1.2		[illegible]	[illegible]	[illegible]
F	1.1	0.9	0.4	[illegible]		1.5	[illegible]	[illegible]
W	0.6	0.5	0.5	0.7		1.3	1.2	[illegible]
G	1.5	0.4	0.6	0.3		0.4	0.7	1.1
A	1.0	0.6	0.4	0.8		1.1	[illegible]	[illegible]
T					1.1			

Fig 26. Use of peptides to analyze mutant constructs and altered conditions for kinase assay

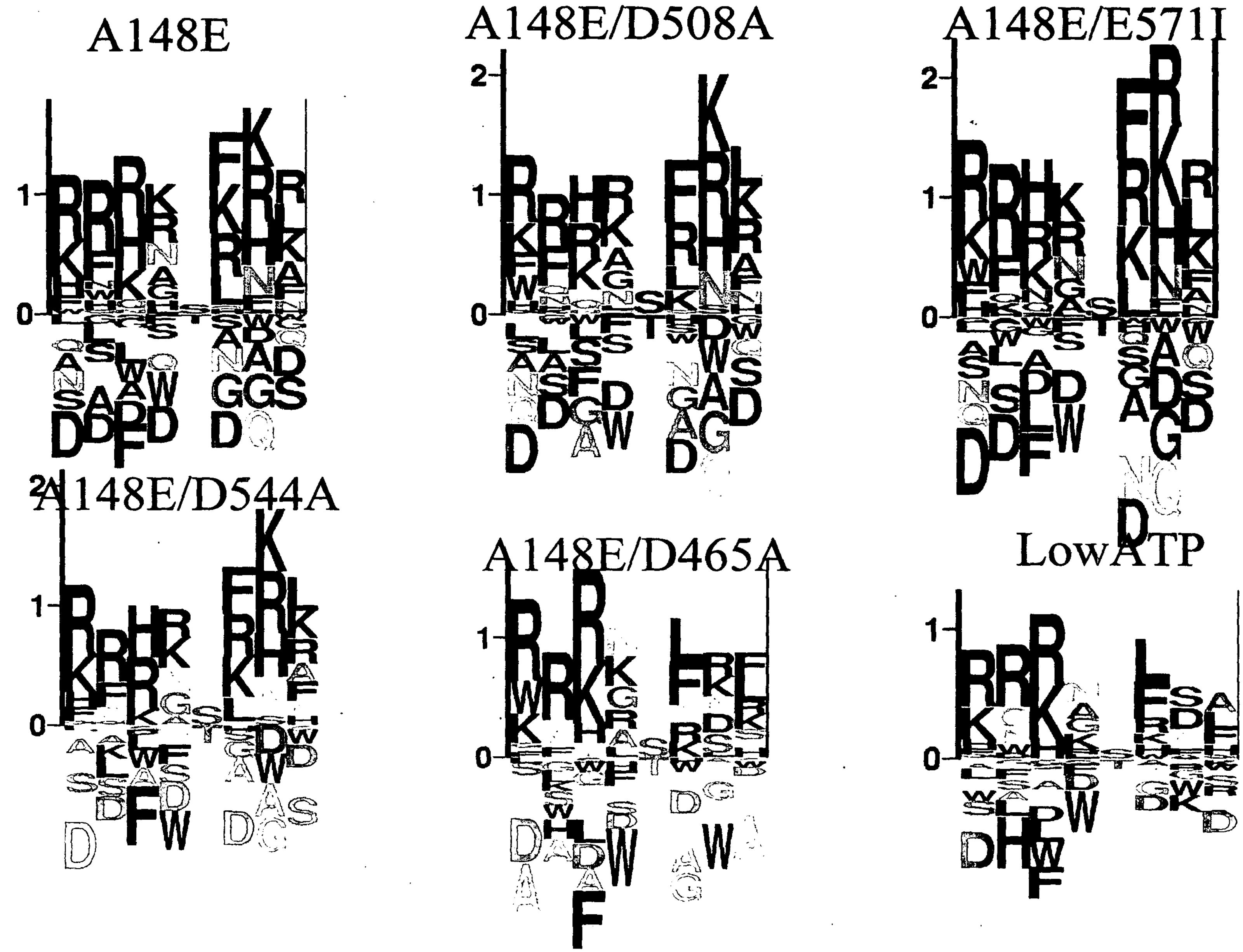


Fig 27. Details of changes observed with mutant constructs and altered conditions for kinase assay

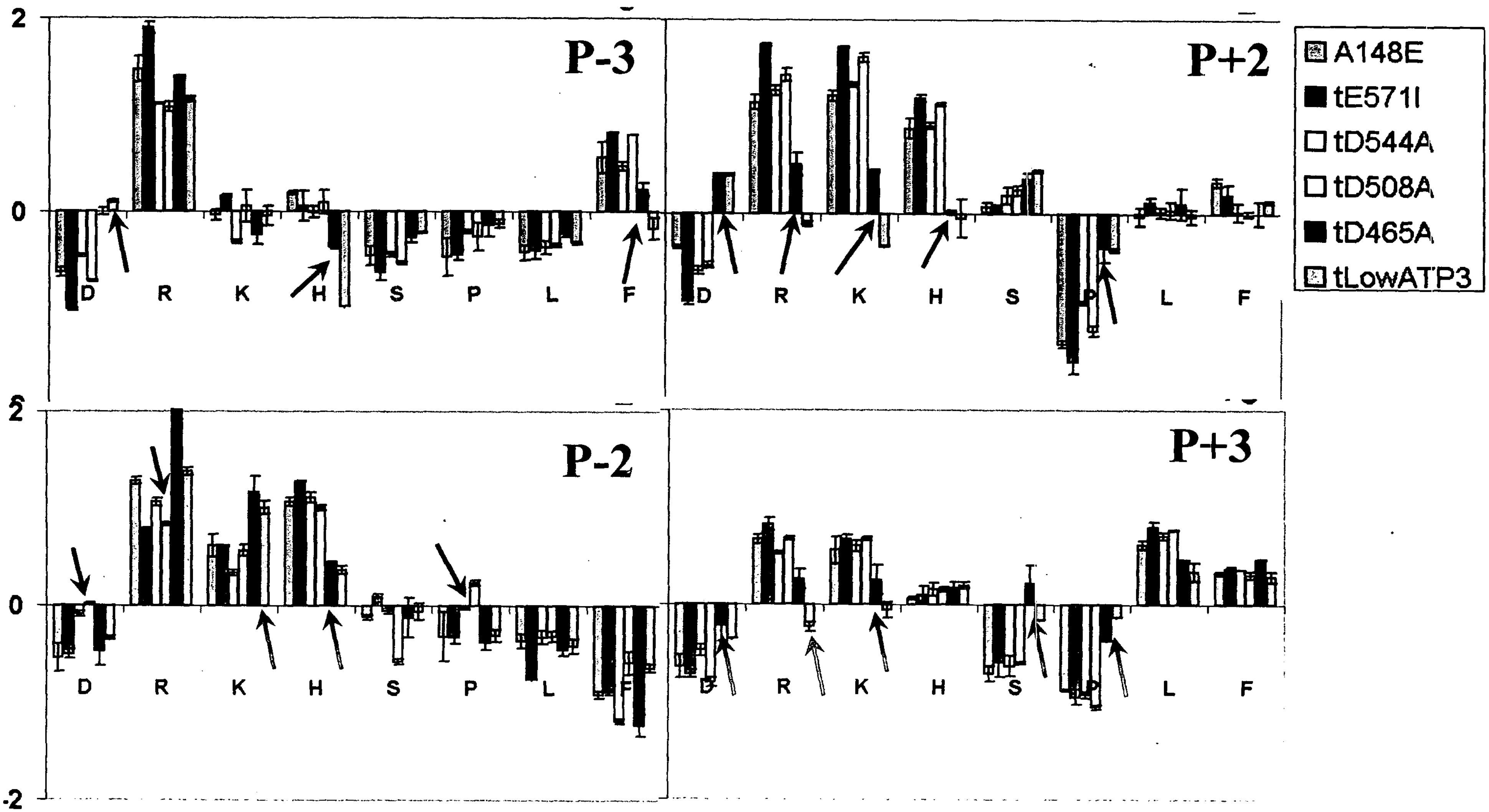


FIG. 28 details of residue references for PKC-theta depend on anchor and phosphorylation residues

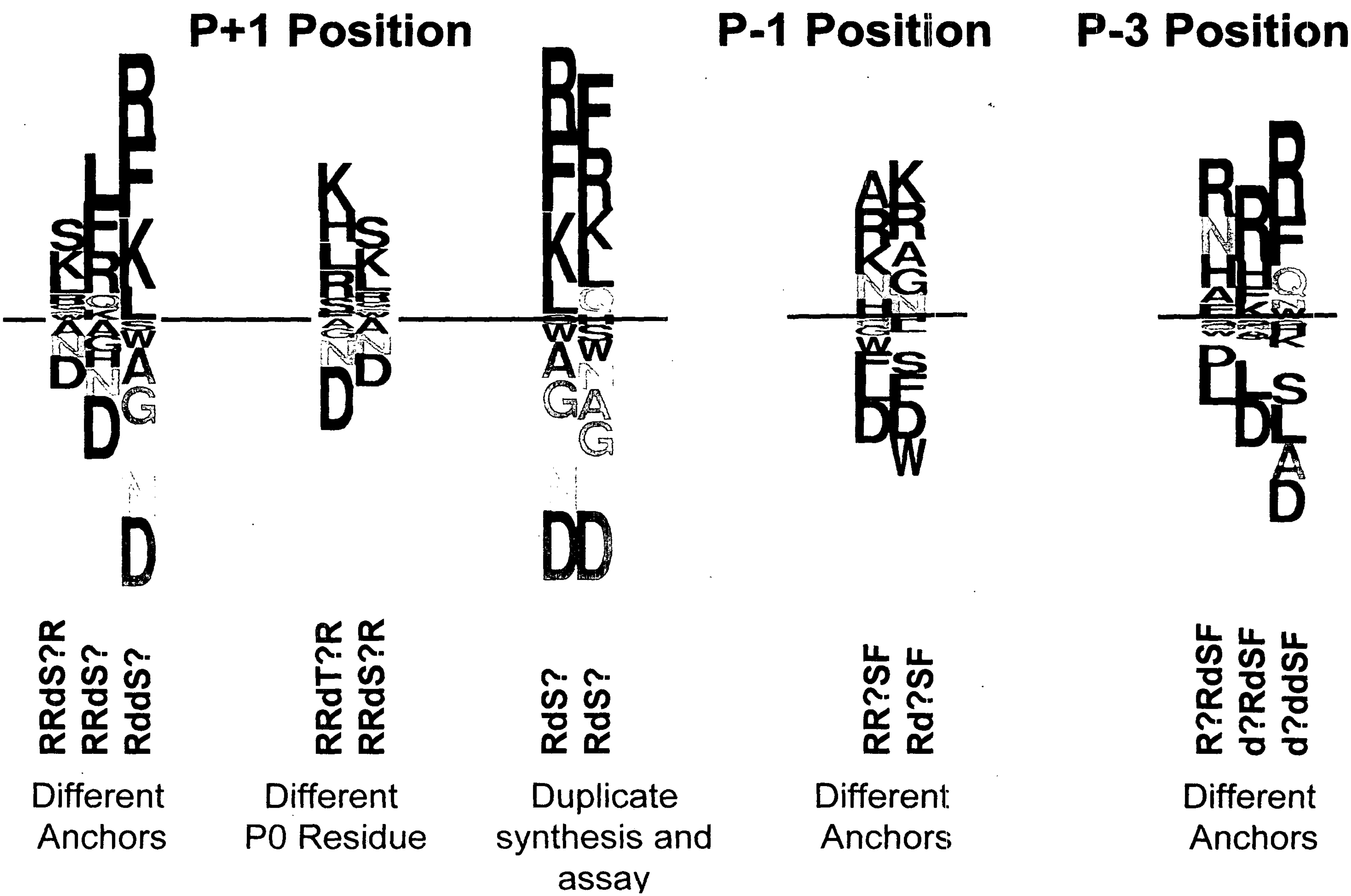


FIG. 29 Results for ROK-alpha with test sets based on ??R??T???? with only 4 query residues

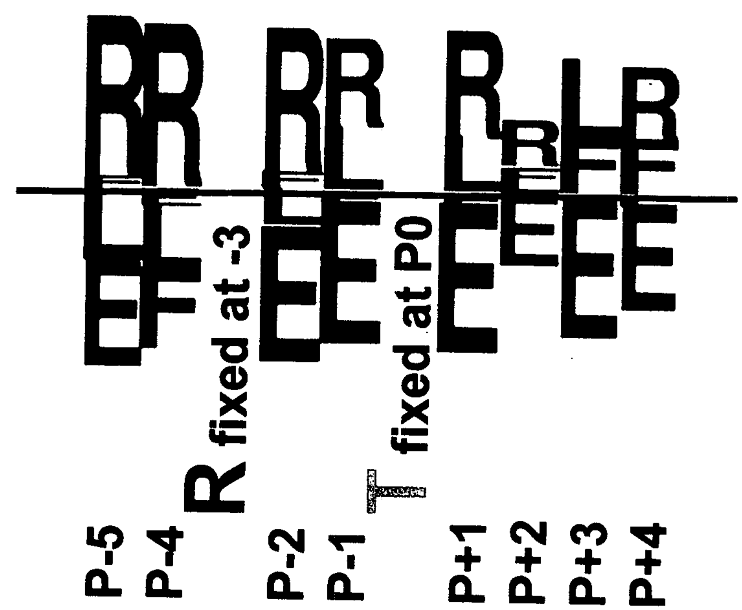


FIG. 30. Details of the R-Pair Anchor optimization set

Position of 1st R	Position of 2nd R: −6	−5	−4	−3	−2
−7	RRddddddSddd	RdRddddSddd	RddRdddSddd	RdddRddSddd	RddddRdSddd
−6		dRRddddSddd	dRdRdddSddd	dRddRddSddd	dRdddRdSddd
−5			ddRRdddSddd	ddRdRddSddd	ddRddRdSddd
−4				dddRRddSddd	dddRdRdSddd
−3					ddddRRdSddd

Position of 1st R	Position of 2nd R: −1	+1	+2	+3
−7	RdddddRSddd	RddddddSRdd	RddddddSdRd	RddddddSddR
−6	dRddddRSddd	dRdddddSRdd	dRdddddSdRd	dRdddddSddR
−5	ddRdddRSddd	ddRddddSRdd	ddRddddSdRd	ddRddddSddR
−4	dddRddRSddd	dddRdddSRdd	dddRdddSdRd	dddRdddSddR
−3	ddddRdRSddd	ddddRddSRdd	ddddRddSdRd	ddddRddSddR
−2	dddddRRSddd	dddddRdSRdd	dddddRdSdRd	dddddRdSddR
−1		ddddddRSRdd	ddddddRSdRd	ddddddRSddR
+1			dddddddSRRd	dddddddSRdR
+2				dddddddSdRR

FIG. 31: R-Pair set results for PKA

Position of 1st R	Position of 2nd R: -7	-6	-5	-4	-3	-2	-1	+1	+2	+3
-7		[illegible]	0.3	-0.3	[illegible]	[illegible]	-0.8	-0.8	-0.4	-1.4
-6	[illegible]		[illegible]	0.4	[illegible]	[illegible]	-0.5	0.1	-1.6	-0.7
-5	0.3	[illegible]		[illegible]	[illegible]	[illegible]	-0.4	-0.8	0.4	-0.9
-4	-0.3	0.4	[illegible]		[illegible]	[illegible]	-0.5	-0.5	-0.9	-1.0
-3	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
-2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	-0.2	0.3	-0.1
-1	-0.8	-0.5	-0.4	-0.5	[illegible]	[illegible]		[illegible]	-1.4	-1.5
+1	-0.8	0.1	-0.8	-0.5	[illegible]	-0.2	[illegible]		-0.2	-1.9
+2	-0.4	-1.6	0.4	-0.9	[illegible]	0.3	-1.4	-0.2		-2.8
+3	-1.4	-0.7	-0.9	-1.0	[illegible]	-0.1	-1.5	-1.9	-2.8	
avg	0.0	0.2	0.3	0.1	[illegible]	[illegible]	-0.2	-0.3	-0.5	-1.1

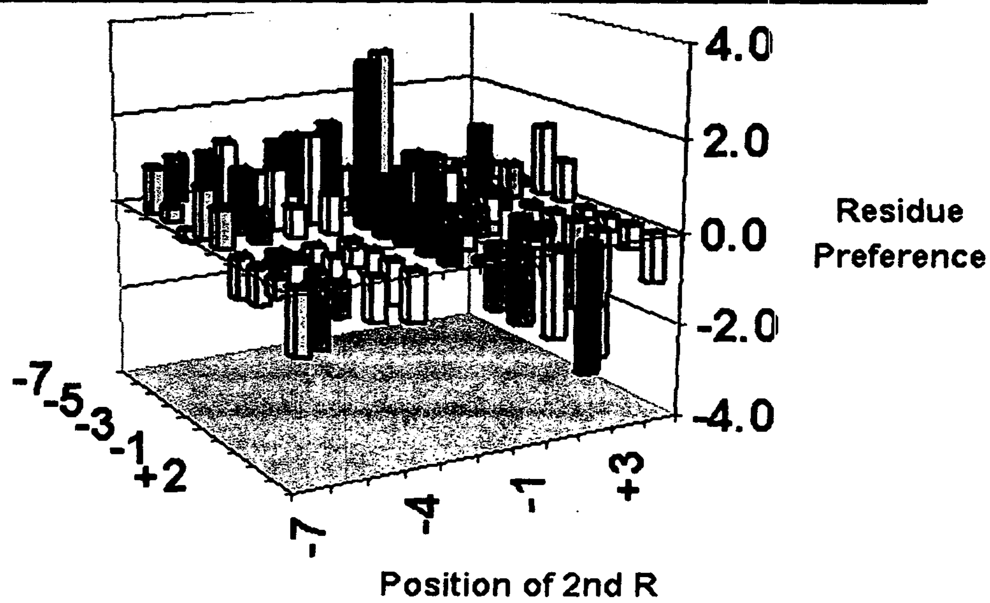


FIG. 32: R-Pair set reveals positions associated with the high preference for R

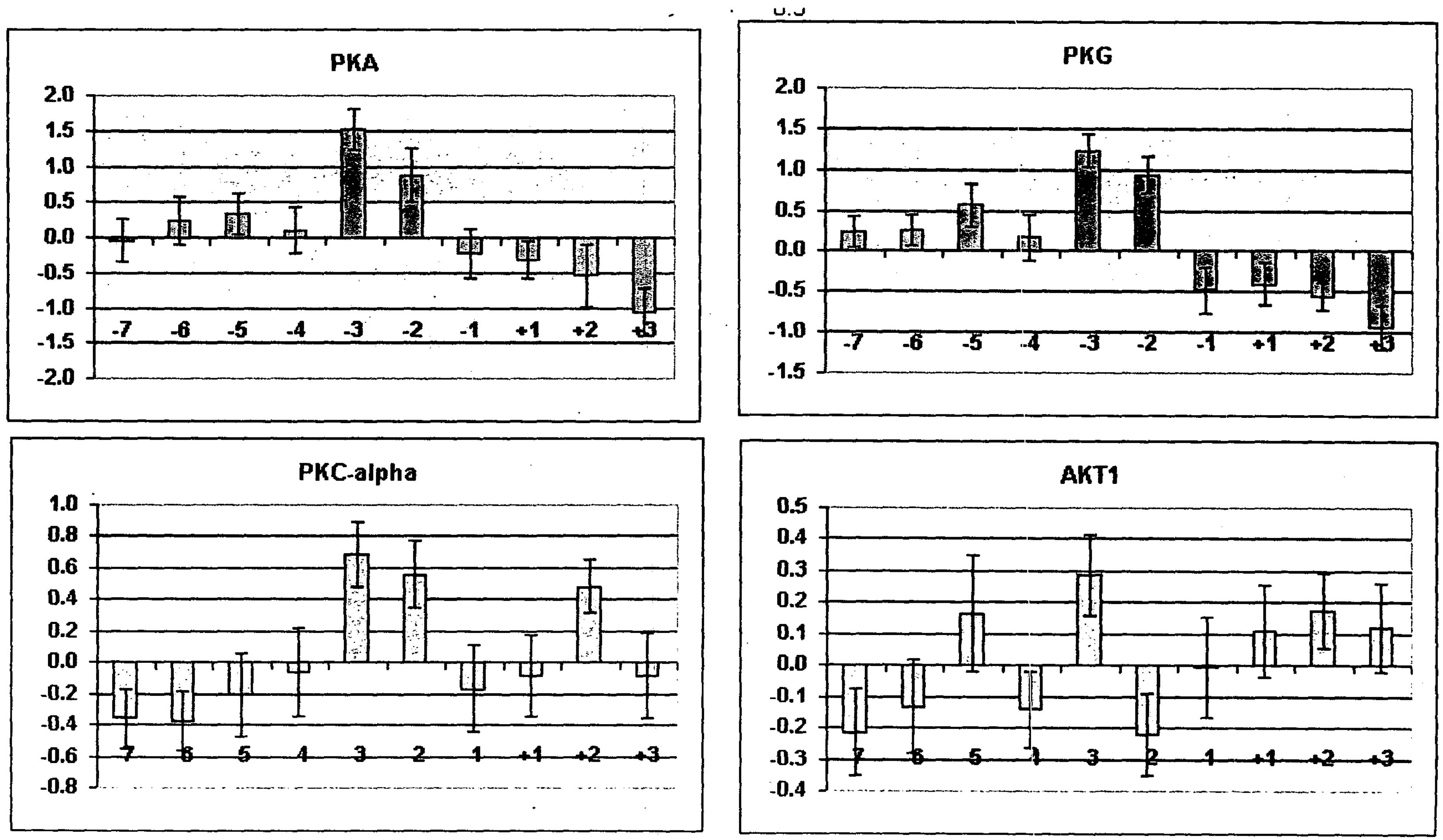


FIG. 33 : Detection of specific phosphorylation of LIMK-2 by Western blot with the pPKC antibody which is augmented following stimulation by the T-cell receptor

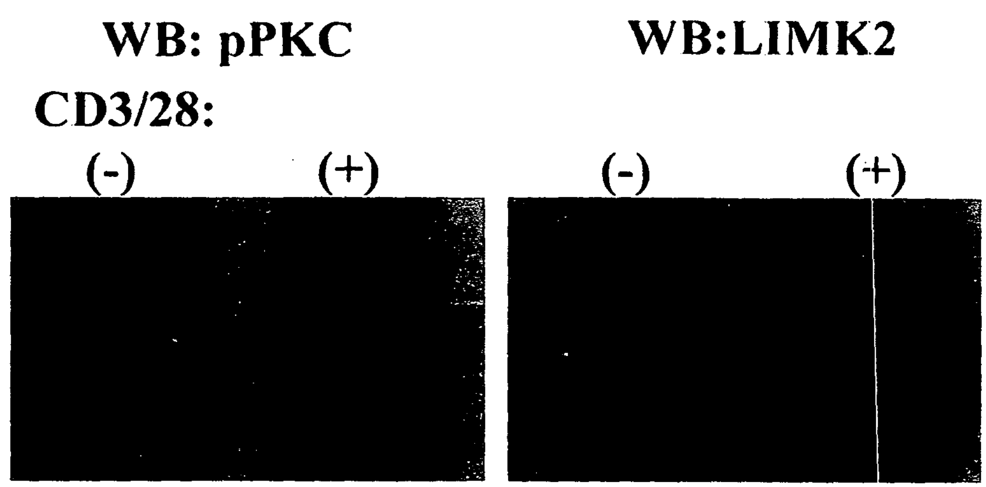


FIG. 34 : Detection of phosphorylation of MLK3 by Western blot with pPKC antibody

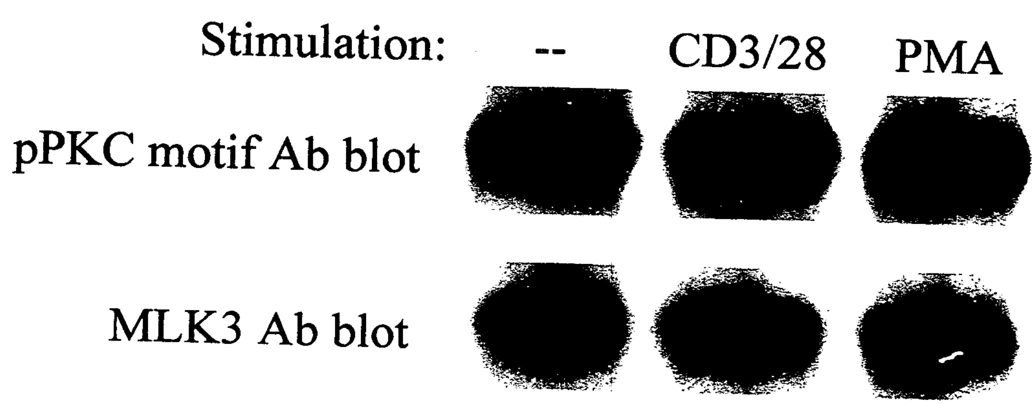


FIG. 35 Diagram of a computerized system in conjunction with which embodiments of the invention may be implemented